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14. ABSTRACT
Organisms have adapted to the relentless cycles of night and day by evolving an internal timekeeping system called the circadian clock, which induces healthy rhythms of rest and activity in synchrony with the earth's rotation. Adverse health and cognitive effects are incurred when this synchrony is chronically disrupted. However, the mechanisms by which these molecular clocks oscillate and transduce timing signals are unclear in any organism. The goal of this equipment grant is to support research in the lab of Andy LiWang, whose objective is to elucidate the molecular mechanism of biological timekeeping. Funds from this grant were used to purchase several pieces of

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a. REPORT	b. ABSTRACT	c. THIS PAGE			Andy LiWang
UU	UU	UU	UU		19b. TELEPHONE NUMBER 209-777-6341

Report Title

Final Report: HBCU Equipment for AFOSR Project 13RSL012: The Mechanism by which ADP Regulates the Structure and Function of the Protein KaiC

ABSTRACT

Organisms have adapted to the relentless cycles of night and day by evolving an internal timekeeping system called the circadian clock, which induces healthy rhythms of rest and activity in synchrony with the earth's rotation. Adverse health and cognitive effects are incurred when this synchrony is chronically disrupted. However, the mechanisms by which these molecular clocks oscillate and transduce timing signals are unclear in any organism. The goal of this equipment grant is to support research in the lab of Andy LiWang, whose objective is to elucidate the molecular mechanism of biological timekeeping. Funds from this grant were used to purchase several pieces of instrumentation that are being used to measure interactions between circadian clock proteins, in order to understand how their interactions produce biochemical rhythms that match sunrise and sunset. Detailed knowledge of clock protein-protein interactions at the structural and thermodynamic levels, and under non-equilibrium conditions is expected to allow the LiWang lab to develop predictive models of clock behavior. The United States military is expected to benefit from this research, because it could lead to new strategies with which to minimize the negative effects of circadian disruption on its personnel as they are deployed across different time zones.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
07/25/2014	1.00	Roger Tseng, Yong-Gang Chang, Ian Bravo, Robert Latham, Abdullah Chaudhary, Nai-Wei Kuo, Andy LiWang. Cooperative KaiA–KaiB–KaiC Interactions Affect KaiB/SasA Competition in the Circadian Clock of Cyanobacteria, Journal of Molecular Biology, (01 2014): 389. doi: 10.1016/j.jmb.2013.09.040
TOTAL:	**1**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received		Paper
TOTAL:		

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper	
07/25/2014	2.00	Yong-Gang Chang, Roger Tseng, Andy LiWang. Protein Flexibility and Gymnastics Drive Robust Clockwise Ticking of a Three-Protein KaiABC Oscillator, The 28th Annual Symposium of the Protein Society. 27-JUL-14, . : ,

TOTAL: **1**

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper	
05/18/2015	3.00	Yong-Gang Chang, Susan E. Cohen, Connie Phong, William K. Myers, Yong-Ick Kim, Roger Tseng, Jenny Lin, Li Zhang, Joseph S. Boyd, Yvonne Lee, Shannon Kang, David Lee, Sheng Li, R. David Britt, Michael J. Rust, Susan S. Golden, Andy LiWang. Protein Fold Switching is the Linchpin Joining the Circadian Oscillator to Clock Output in Cyanobacteria, Science (08 2014)

TOTAL: **1**

Number of Manuscripts:

Books

Received Book

TOTAL:

Received Book Chapter

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Roger Tseng	0.00	
Joel Heisler	0.00	
Antonios Chionis	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**3**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Yong-Gang Chang	0.00
Archana Chavan	0.00
FTE Equivalent:	**0.00**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Andy LiWang	0.00	
FTE Equivalent:	**0.00**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED	Discipline
Yassamin Ihat	0.00	Human biology
Montana Altman	0.00	Human biology
Michael Montoya	0.00	Human biology
FTE Equivalent:	**0.00**	
Total Number:	**3**	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period: 0.00

The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:..... 0.00

Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):..... 0.00

Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:..... 0.00

The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense 0.00

The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields: 0.00

Names of Personnel receiving masters degrees

NAME
Total Number:

Names of personnel receiving PHDs

NAME
Total Number:

Names of other research staff

NAME	PERCENT_SUPPORTED

FTE Equivalent:
Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

Technology Transfer

Scientific Progress and Accomplishments

Organisms are adapted to the relentless cycles of day and night, because they evolved timekeeping systems called circadian clocks, which regulate biological activities with ~24-h rhythms [1] . Chronic disruption of circadian rhythms degrades cognitive skills, motivation, performance, reaction time, alertness, memory, judgment, and mood [2-4] , as well as increases the risk of obesity [5-7] , diabetes [8, 9] , heart disease [10] , and cancer [11, 12] . Our lab studies the circadian clock of a model organism to elucidate mechanisms by which circadian rhythms are generated. A three-protein oscillator comprised of KaiA, KaiB, and KaiC, which together generate a circadian (~24 h) rhythm of KaiC autophosphorylation, drives the clock of cyanobacteria [13, 14] . We have discovered that KaiB flips between two distinct three-dimensional folds, gsKaiB ⇔ fsKaiB, and its rare metamorphosis to the active fsKaiB state provides a time delay that is required to match the oscillator's period to the earth's rotation (**Fig. 1**). Once KaiB switches folds, it binds KaiC and captures KaiA, initiating KaiC autodephosphorylation, and regulates components of the clock-output pathway, CikA and SasA [15-20] , providing the link that joins the timekeeping and signaling functions of the oscillator. Intrinsically rare excursions of KaiB between two distinct folds provides an elegant mechanism by which the oscillator can temporally separate opposing phases of the clock, ensure a correct period, and reciprocally regulate mutually antagonistic output-signaling pathways. Our findings as summarized in **Fig. 1** are now in press at the journal *Science*.

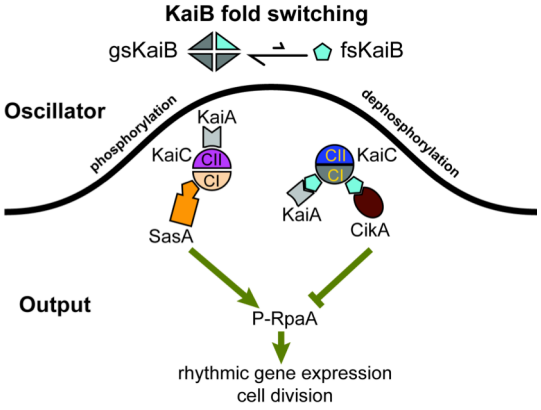

Figure 1. Excursion of KaiB to the rare fold-switch fsKaiB state causes it to displace SasA for binding to KaiC. KaiC-stabilized fsKaiB captures KaiA, initiating the dephosphorylation phase of the cycle. These aspects control oscillator period. CikA and KaiA compete for binding to fsKaiB, further linking oscillator function related to KaiA and output activity via CikA-mediated dephosphorylation of RpaA. The competitive interactions of fsKaiB with SasA, and KaiA with CikA, implicate "output components" CikA and SasA as parts of an extended oscillator.

Currently, we are working to resolve the following questions:

- In order to understand why KaiB switches folds on the time scale of the earth's rotation, we want to figure out the pathway by which KaiB switches folds, and the energetics of fold switching.
- We have evidence that the active fsKaiB state is increasingly favored as the temperature is decreased. Thus, we want to determine the extent to which KaiB fold switching helps the clock maintain a circadian period at different temperatures. Temperature compensation is a defining criterion of circadian clocks, but its mechanism remains unclear.
- The slowness by which KaiC autophosphorylates and autodephosphorylates (**Fig. 1**) is an essential pacesetter for the cyanobacterial clock. Therefore, we are endeavoring to determine the mechanism by which these rates are set.

Findings from our lab are expected to help the U.S. military develop new strategies with which to reduce the negative effects of circadian disruption on its personnel as they are deployed across different time zones.

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